

enhanced intracellular delivery, they have simultaneously introduced new disadvantages of their own. Thus, both methods exhibit some carrier cytotoxicity, and like other protocols, neither strategy allows for any tissue or cell targeting. In short, intracellular delivery and tissue specificity remain major obstacles to the implementation of antisense drugs in the treatment of human disorders.

Other techniques for the delivery of oligonucleotides to cells include the use of: (a) folate-PEG-liposome constructs for the delivery of antisense DNA against growth factor receptor (Wang et al., 1995); (b) folic acid-polylysine constructs for the delivery of c-myc antisense DNA (Ginobbi et al., (1997); (c) tris(N-acetylgalactosamine aminohexyl glycoside) amide of tyrosyl(glutamyl)-glutamate (YEE(GalNAcAH)<sub>3</sub>) linked to polylysine for the delivery of DNA to cells via the asialoglycoprotein receptor (Merwin et al., 1994); and (d) water-soluble block polycations (Kabanov et al., 1995).

It has been known for some time that a pharmaceutically active agent can be attached to a carrier or molecule. The term "prodrug" is often associated with such systems wherein the active agent is bonded to another molecule for purposes of administration. The drug is usually inactive in the prodrug state and the bond is later cleaved releasing the drug at a site where it can be effective. However, such systems are not as useful as might be desired for various reasons, site specificity being one. Also, the release of the drug from its carrier requires the presence of some agent or event to separate the active drug from its carrier or molecule and, as such, may rely on factors such as the presence of a specific enzyme, pH conditions, time release and the like, which may be variable from host to host and which may not be effectively implemented.

For example, transmembrane transport of nutrient molecules is a critical cellular function. Because practitioners have recognized the importance of transmembrane transport to many areas of medical and biological science, including drug therapy, peptide therapy and gene transfer, there have been significant research efforts directed to the understanding and application of such processes. Thus, for example, transmembrane delivery of nucleic acids has been encouraged through the use of protein carriers, antibody carriers, liposomal delivery systems, electroporation, direct injection, cell fusion, viral carriers, osmotic shock, and calcium-phosphate mediated transformation. However, many of those techniques are limited both by the types of cells in which transmembrane transport is enabled and by the conditions of use for successful transmembrane transport of exogenous molecular species. Further, many of these

known techniques are limited in the type and size of exogenous molecule that can be transported across a membrane without loss of bioactivity.

One method for transmembrane delivery of exogenous molecules having a wide applicability is based on the mechanism of receptor-mediated endocytotic activity. Unlike many other methods, receptor-mediated endocytotic activity can be used successfully both *in vivo* and *in vitro*. Receptor-mediated endocytosis involves the movement of ligands bound to membrane receptors into the interior of an area bounded by the membrane through invagination of the membrane. The process is initiated or activated by the binding of a receptor-specific ligand to the receptor. Many receptor-mediated endocytotic systems have been characterized, including those recognizing galactose, mannose, mannose 6-phosphate, transferrin, asialoglycoprotein, transcobalamin (Vitamin B<sub>12</sub>),  $\alpha$ -2-macroglobulins, insulin, and other peptide growth factors such as epidermal growth factor (EGF).

Receptor-mediated endocytotic activity has been utilized for delivering exogenous molecules such as proteins and nucleic acids to cells. Generally, a specified ligand is chemically conjugated by covalent, ionic or hydrogen bonding to an exogenous molecule of interest (i.e. the exogenous compound), forming a conjugate molecule having a moiety (the ligand portion) that is still recognized in the conjugate by a target receptor. Using this technique, the phototoxic agent psoralen has been conjugated to insulin and internalized by the insulin receptor endocytotic pathway (Gasparro, 1986); the hepatocyte-specific receptor for galactose terminal asialoglycoproteins has been utilized for the hepatocyte-specific transmembrane delivery of asialoorosomucoid-poly-L-lysine non-covalently complexed to a DNA plasmid (Wu, 1987); the cell receptor for epidermal growth factor has been utilized to deliver polynucleotides covalently linked to EGF to the cell interior (Myers, 1988); the intestinally situated cellular receptor for the organometallic Vitamin B<sub>12</sub>-intrinsic factor complex has been used to mediate delivery to the circulatory system of a vertebrate host a drug, hormone, bioactive peptide or immunogen complexed with Vitamin B<sub>12</sub> and delivered to the intestine through oral administration (Russell-Jones et al., 1995); the mannose-6-phosphate receptor has been used to deliver low density lipoproteins to cells (Murray and Neville, 1980); the cholera toxin binding subunit receptor has been used to deliver insulin to cells lacking insulin receptors (Roth and Maddox, 1983); the human chorionic gonadotropin receptor has been employed to deliver a ricin a-chain coupled to HCG to cells with the appropriate HCG receptor in order to kill the cells (Oeltmann and Heath,

1979); the transferrin receptor has been used to deliver mitomycin C to sarcoma cells (Tanaka et al., 1996) or to deliver doxorubicin to multidrug-resistant cells (Fritzer et al., 1996); the biotin receptor has been employed to deliver hypoxanthine-guanine phosphoribosyl transferase (HGPRT) by biotinylation the HGPRT to restore growth to HGPRT deficient cells (Low et al., 1995); and the folic acid receptor has been used to deliver antisense DNA to src-transformed fibroblast cells (Low et al., 1995).

Russell-Jones et al. (1995), describes a system which involves the formation of a covalent bond between the pharmaceutical agent one wishes to deliver and a modified Vitamin B<sub>12</sub> to form a conjugate molecule. The conjugate is orally administered and is then transported from the intestinal lumen to the circulation. Importantly, the pharmaceutical agent and the vitamin are bound through an amide linkage which is prone to acid hydrolysis. Russell-Jones et al. found that many biologically active pharmaceutical agents can be bound to B<sub>12</sub> for facilitating the introduction of the drug into the blood stream through oral administration. Importantly, no method was provided whereby the drug-B<sub>12</sub> bond could be selectively cleaved, nor could location of the active pharmaceutical agent be controlled once activated. Instead, Russell-Jones et al. relied on biochemical degradation of the drug-B<sub>12</sub> bond to release the drug in its active form. Importantly, under this method the drug could be released in its active form anywhere within the circulation system, diminishing the importance of the active transport of B<sub>12</sub> into cancer tissue. Moreover, the conjugates formed under this method require the modification of the structure of the corrin ring of the B<sub>12</sub> molecule, which modification can have serious effects on receptor interactions.

Thus, there exists a need for a drug delivery system which can be utilized for the delivery of bioactive agents, including pharmaceuticals, peptides and oligonucleotides. There is also a need for a drug delivery system which can be used for site-specific release of the bioactive agent in the cells, tissues, or organs in which a therapeutic effect is desired to be effected.

#### SUMMARY OF THE INVENTION

The present invention relates to bioconjugates and the delivery of bioactive agents which are preferably targeted for site specific release in cells, tissues or organs. More particularly, this invention relates to bioconjugates which comprise a bioactive agent and an organocobalt complex. The bioactive agent is covalently bonded directly or indirectly to the cobalt atom of